Historic, archived document

Do not assume content reflects current scientific knowledge, policies, or practices.

164.1
R31A
Cop. 2

U. S. DEPT. OF AGRICULTURE
LIBRARY
MAY 10 1963
CURRENT SERIAL RECORDS

CROPS RESEARCH

ARS 34-44
March 1963

Saponin Content of Alfalfa

as related to

- LOCATION
- CUTTING
- VARIETY
- OTHER VARIABLES

Agricultural Research Service
U.S. DEPARTMENT OF AGRICULTURE

The experiment described in this publication was designed to obtain information on the magnitude and nature of variation in saponin content of alfalfa. The results reported are a joint contribution from the Crops Research Division and the Western Utilization Research and Development Division, Agricultural Research Service, U.S. Department of Agriculture, in cooperation with the California, Iowa, Kansas, Nebraska, New York, North Carolina, Oregon, and Utah Agricultural Experiment Stations.

Credit is due M. T. O'Sullivan, General Chemist, Western Utilization Research and Development Division; and R. R. Hill, Jr., Research Agronomist, Crops Research Division, Agricultural Research Service, U.S. Department of Agriculture; Albany, Calif., and Ithaca, N. Y., respectively; for assisting with chemical and statistical analyses, respectively.

CONTENTS

	Page
Importance of saponins in feed	1
Chemical nature of saponins	1
Objectives of study	2
Materials and methods	2
Planting and harvesting procedures	2
Chemical procedures	2
Statistical procedures	5
Results	7
Combined analysis of saponin data from eight locations	7
Varieties and cuttings not common to all locations	8
Relationship of saponin content to yield, defoliation, and other chemical constituents	10
Discussion	13
Summary	14
Literature cited	15
Appendix	17

SAPONIN CONTENT OF ALFALFA AS RELATED TO LOCATION, CUTTING, VARIETY, AND OTHER VARIABLES [1]

C. H. Hanson, G. O. Kohler, J. W. Dudley, E. L. Sorensen, G. R. Van Atta, K. W. Taylor, M. W. Pedersen, H. L. Carnahan, C. P. Wilsie, W. R. Kehr, C. C. Lowe, E. H. Standford, and J. A. Yungen[2]

IMPORTANCE OF SAPONINS IN FEED

Saponins are glycosides that occur in a wide variety of plants. Isolated saponins are white crystalline or amorphous solids having a bitter taste and an irritating effect on mucous membranes. They have a wide variety of physiological effects, mostly adverse, on animals, so saponins are considered deleterious (14).[3] Their deleterious effects on animals include: Toxicity to fish and snails (14); hemolytic effects upon injection (14); inhibition of the normal rhythmical contraction of smooth muscle (7); and inhibition of respiration of animal tissues (12).

Alfalfa saponins, which belong to the class of triterpenoid saponins, are of practical importance since it has been shown that they are probably involved in pasture bloat in cattle and sheep (7). Purified alfalfa saponins inhibit growth in young chickens (2, 5, 10) and depress egg production in laying hens (2, 6). The effects from alfalfa meal are small because it usually is used in rations at low levels as a vitamin and protein supplement. Low saponin alfalfa would be desirable since saponins have no known value in livestock feed and, under some conditions, may have deleterious effects.

CHEMICAL NATURE OF SAPONINS

Although considerable work has been done on the chemistry of the alfalfa saponins, progress has been slow since the crude saponin isolated is complex in nature, and its components are extremely difficult to separate (3, 15). Upon hydrolysis of the mixed saponin, a mixture of triterpenoid sapogenins, sugars, and uronic acids was obtained. Among the sugars identified were glucose, arabinose, and xylose. Galacturonic acid, galactose, and rhamnose were also found, but in lesser quantities.[4] It was shown by isolation and by column and paper chromatography that the sapogenin mixture contained a number of components (11, 15). Of these, four were isolated and identified, or characterized. These were soya-sapogenols A, B, and C and medacagenic acid (4, 11, 17). A fifth, lucernic acid, was isolated and partially characterized but the complete structure is not yet known (8).

The similarity of properties of these saponin components complicates their separation, but

[1] Joint contribution from Crops Research Division, and Western Utilization Research and Development Division, ARS, USDA, in cooperation with the California, Iowa, Kansas, Nebraska, New York, North Carolina, Oregon, and Utah Agricultural Experiment Stations.

[2] Respectively, Research Agronomist, CRD, ARS, USDA, Beltsville, Md.; Chief, Field Crops Lab., WURDD, ARS, USDA, Albany, Calif.; Geneticist, CRD, ARS, USDA, Raleigh, N.C.; Research Agronomist, CRD, ARS, USDA, Manhattan, Kans.; Chemist, Field Crops Lab., WURDD, ARS, USDA, Albany, Calif.; General Engineer, Field Crops Lab., WURDD, ARS, USDA, Albany, Calif.; Research Agronomist, CRD, ARS, USDA, Logan, Utah; Research Agronomist, CRD, ARS, USDA, Reno, Nev., formerly, U.S. Regional Pasture Research Lab., University Park, Pa.; Professor of Agronomy, Iowa State University, Ames, Iowa; Research Agronomist, CRD, ARS, USDA, Loncoln, Nebr.; Associate Professor of Plant Breeding, Cornell University, Ithaca, N.Y.; Professor of Agronomy, University of California, Davis; Assistant Agronomist, Oregon State College, Medford, Oreg.

[3] Figures in parentheses refer to Literature Cited at end of this publication.

[4] Van Atta, G. R. Unpublished data.

by the same token has made it possible to evolve a method of analysis which measures the entire complex (16).[5] This method was used in the present study.

OBJECTIVES OF STUDY

This experiment was designed to obtain information on the magnitude and nature of variation in saponin content of alfalfa. Some of the questions on which information was sought were: Do varieties differ in saponin content? What is the relative importance of varieties, cuttings, locations, and their interactions with respect to fluctuation of saponin content? Is saponin content associated with yield or defoliation? To what extent is saponin content associated with protein, crude fiber, fat. ash, and nitrogen-free extract? Would breeding for low saponin content be a practical objective?

MATERIALS AND METHODS

Planting and Harvesting Procedures

The main part of this investigation was concerned with performance and chemical data from the first three cuttings of four alfalfa varieties sampled in 1959 at each of eight locations. The varieties Buffalo, Lahontan, Ranger, and Vernal were chosen as being representative of the germ plasm found in principal alfalfa varieties grown in the United States. The locations were Ithaca, N.Y.; University Park, Pa.; Raleigh, N.C.; Ames, Iowa; Lincoln, Nebr.; Manhattan, Kans. (one test each on dryland and irrigated farms); and Logan, Utah. The four varieties, three cuttings, and eight locations constituting the "core" of the experiment on which combined statistical analyses were conducted, and additional varieties and cuttings included at various locations, are indicated in table 1. Plot size and other plot details are given in table 2.

Immediately before harvesting for hay, the plots were scored from 0 to 7 for defoliation. Scores estimated defoliation as follows: 0 = none, 1 = 10 percent, 2 = 20 percent, 3 = 30 percent, 4 = 40 percent, 5 = 50 percent, 6 = 60 percent, and 7 = 70 percent or greater.

Plots were harvested when the alfalfa was in about the 1/10-bloom stage; that is, when either 1/10th of the plants had at least one stem with one or more open florets or when 25 percent of the plants had crown shoots 1/4 to 1/2 inch in height. Cutting height was 3 inches. An attempt was made to collect samples for analysis between 9 a.m. and 12 noon, local standard time. At each location, all varieties were cut on the same date.

At the time plants were cut for hay, a sample of green hay sufficient to provide 40 grams of dry material for chemical analysis was obtained from a plot of each variety in each of 4 replications. Weeds or foreign material were removed before drying. After drying, replications 1 and 2 were composited as were replications 3 and 4 to give 2 replications for chemical analyses. Yield and defoliation data were combined in a similar manner before statistical analyses were made. Temperatures used for drying the samples harvested for chemical analysis were between 140° and 160° F., except in Nebraska where 110° was used.

Chemical Procedures

Total saponin was determined according to the procedure described by Van Atta and others (16). Percentage of saponin, as determined by the method, represents the total of the several saponins occurring in alfalfa (7, 15) plus a nonsaponin fraction which is not separated from the saponins by the analytical procedure. The proportion of nonsaponins included in the analytical products and weighed as saponins is nearly constant and amounts

[5] Van Atta, G. R. Supplementary Method for Determining Saponins in Alfalfa. Jour. Agr. and Food Chem. [In press.]

TABLE 1.--Alfalfa varieties and locations (indicated by x's), and cuttings on which agronomic and chemical data were obtained[1]

Variety	N.Y.	Pa.	N.C.	Iowa	Nebr.	Kans. (dry-kabd)	Kans. (irri-gated)	Utah	Oreg.	Calif.
Buffalo.....	x	x	x	x	x	x	x	x	x	x
Lahontan.....	x	x	x	x	x	x	x	x	x	x
Ranger.....	x	x	x	x	x	x	x	x	x	x
Vernal.....	x	x	x	x	x	x	x	x	x	
African.....			x							x
Du Puits.....						x	x	x		
Tourneur 501...								x		
Caliverde.....										x
Number of cuttings harvested and analyzed	3	3	3	3	3	3	4	3	2	5

[1] Three cuttings of the varieties within rectangle (first three of four in irrigated Kansas test) formed the "core" of the experiment.

TABLE 2.--Soil types, seeding dates, and other field plot information

[Four replications]

State	Soil type	Date seeded	Method of seeding	Plot size (ft.)	Area harvested (sq. ft.)
Iowa.....	Clarion loam	4-14-59	4-row drilled plots	3-1/2 x 15	52.5
Kansas (irrigated-Ashland Farm)	Sarpy fine sandy loam	4-20-57	9-row drilled plots	5 x 20	61.4
Kansas (dryland-Agronomy Farm)	Geary silt loam	4-25-57	9-row drilled plots	5 x 20	61.4
Nebraska.....	Sharpsburg silty clay loam	4-30-59	3-row drilled plots	2-1/2 x 15	37.5
New York.....	Erie	5-21-58	Broadcast	5 x 16	40.6
North Carolina.....	Georgeville silty clay loam	9-1-58	3-row drilled plots	2-1/2 x 15	37.5
Pennsylvania.....	Hagerstown silty clay loam	5-15-58	Broadcast	5 x 20	60.0
Utah.....	Salt Lake silt loam	5-2-57	5-row drilled plots	3-1/6 x 20	63.3
California.....	Yolo fine sandy loam	10-10-59	Broadcast	5 x 20	70.0
Oregon.....	Medford gravelly clay loam	4-9-54	5-row drilled plots	5 x 24	----

to about 25 percent of the total.[6] Hence, the true saponin contents of the samples closely approximate 75 percent of the values reported.

In addition to analysis for saponin content, each sample was subjected to a feed analysis, which included determinations for percentages of protein (N x 6.25), crude fiber, crude fat, ash, and moisture. Nitrogen-free extract was calculated by difference. Methods used for the feed analyses were those of the American Association of Cereal Chemists (1). Each chemical constituent was expressed as percent on a moisture-free basis.

Statistical Procedures

Combined analyses over locations.--A combined analysis of the saponin data from the "core" cuttings, varieties, and locations was made. The expectations of mean squares for this analysis are shown in table 3. All main effects except cuttings were assumed to be random.

Appropriate linear functions of mean squares were used to estimate each variance component. A plot component was computed by adding together all of the components except that for the cuttings main effect. Individual variance components were expressed as a percentage of the plot component. A plot component computed in this manner arises from variation among plots within the same cutting.

In analyses of data for individual locations, all varieties and cuttings were included.

Determining associations between characters.--The sum of squares for each main effect and interaction in the "core" analysis, except those involving replications, were subdivided into the part due to linear regression and the part due to deviations from linear regression for study of the relationship between saponin and other characters. The mean square for deviations from linear regression for each effect was tested with the same mean square that was used for testing the effect. If the deviation from regression mean square was significant, it was used to test the significance of the regression component; if not, the error term used for deviations was used to test the regression component. The test of significance of the mean square for regression is also a test of significance of the correlation coefficient and of the percentage of reduction in sums of squares due to regression (r^2) for the particular effect, since the variance component associated with regression $\beta^2\Sigma x^2$ is equivalent algebraically to $\rho^2\Sigma y^2$. Main effects containing significant interactions not included in any one mean square were tested using the procedure outlined by Snedecor (13, p. 362).

The regression sums of squares for locations, varieties, and cuttings estimate the degree of linear association between saponin and the other characters for the respective sources of variation. The sum of squares due to deviations from linear regression measures the variation in the other characters that is independent of linear association with saponin content, when saponin is used as the "X" variable. Whether saponin is used as the "X" or "Y" variable affects the test of significance, since two variables are seldom measured with the same degree of precision. The percentage of reduction in the sum of squares due to regression will be the same regardless of whether saponin is the "X" or "Y" variable.

The regression sum of squares for interaction effects can be interpreted as measuring the extent to which the interaction effect for saponin varies with the interaction effect for another character.

The regression sums of squares were also obtained for the variety, cutting, and variety x cutting interaction for the individual locations. All varieties and cuttings at individual locations were included in the analyses of individual locations. The regression sum of squares for cuttings for the data from Oregon was not computed because there was only one degree of freedom for cuttings.

[6] See footnote 5.

TABLE 3.--Expected mean squares for the analysis of variance of percentage of saponin in alfalfa hay from four varieties, three cuttings, and eight locations[1]

Source	df	Expected mean square[2]
Locations (L).....	7	$\sigma_e^2 + 3\sigma_{VR(L)}^2 + 12\sigma_{R(L)}^2 + 6\sigma_{LV}^2 + 24\sigma_L^2$
Replications in Locations (R(L))	8	$\sigma_e^2 + 3\sigma_{VR(L)}^2 + 12\sigma_{R(L)}^2$
Varieties (V).....	3	$\sigma_e^2 + 3\sigma_{VR(L)}^2 + 6\sigma_{LV}^2 + 48\sigma_V^2$
V x L.....	21	$\sigma_e^2 + 3\sigma_{VR(L)}^2 + 6\sigma_{LV}^2$
V x R(L).....	24	$\sigma_e^2 + 3\sigma_{VR(L)}^2$
Cuttings (C).....	2	$\sigma_e^2 + 2\sigma_{CVL}^2 + 16\sigma_{CV}^2 + 8\sigma_{LC}^2 + 64\Sigma C_i^2$
C x L.....	14	$\sigma_e^2 + 2\sigma_{CVL}^2 + 8\sigma_{LC}^2$
C x V.....	6	$\sigma_e^2 + 2\sigma_{CVL}^2 + 16\sigma_{CV}^2$
C x V x L.....	42	$\sigma_e^2 + 2\sigma_{CVL}^2$
Error.....	64	σ_e^2
Total.....	191	

[1] Cuttings were assumed to be fixed.

[2] Effects measured by different components of variance: σ_e^2 = error; σ_{CVL}^2 = interaction of cuttings x varieties x locations; σ_{CV}^2 = interaction of cuttings x varieties; σ_{LC}^2 = interaction of locations x cuttings; ΣC_i^2 = fixed cutting effect; $\sigma_{VR(L)}^2$ = interaction of varieties x replications in locations; σ_{VL}^2 = interaction of varieties x locations; σ_V^2 = variety main effect; $\sigma_{R(L)}^2$ = replications in locations main effect; σ_L^2 = location main effect.

Combined Analysis of Saponin Data from Eight Locations

Mean squares.--Differences between locations, varieties, and cuttings, and the interactions of varieties x locations, cuttings x locations, and cuttings x varieties x locations were significant at the 1-percent level for percentage of saponin. Other interactions were not significantly different from zero (table 4, column 3).

Variance component estimates.--The components making significant contributions to plot variation within cuttings fall into roughly three classes (table 5) with cuttings x locations contributing most (36.7 percent); main effects of locations and varieties contributing the next greatest amounts (18.4 and 16.4 percent, respectively); and cuttings x varieties x locations, error, and locations x varieties contributing the least (10.0, 11.4, and 6.0 percent, respectively). Since the cutting effect was assumed to be fixed, it was not included in the plot component. However, the cutting effect was greater than that of any other variable.

Means for saponin content.--For the average of the eight locations in the "core" of the study, Lahontan was lowest in saponin content followed by Ranger, Buffalo, and Vernal in that order (table 6, column 10). Labontan was consistently the lowest ranked variety at all locations. The ranking of the other three varieties varied with location, thus accounting for the significant variety x location interaction.

The first cutting was lower in saponin content than the other two cuttings at all locations except North Carolina and Utah,

TABLE 4.--Mean squares from the combined analysis of variance of percentage of saponin in alfalfa

Source of variation	df	Mean square
Locations	7	0.7255**
Replications in Locations (R in L)	8	.0284
Varieties (V)	3	1.4297**
V x L	21	.0770**
V x R in L	24	.0151
Cuttings (C)	2	3.7909**
C x L	14	.5589**
C x V	6	.0654
C x V x L	42	.0541**
Error	64	.0196

** = significant at the 1-percent level.

TABLE 5.--Variance component estimates from combined analysis for percentage of saponin in alfalfa

Component	Estimate	Estimate expressed as percentage of σ^2_{plot}[1]
σ^2_L	0.0316	18.4
$\sigma^2_{R(L)}$	.0011	.6
σ^2_V	.0282	16.4
σ^2_{LV}	.0103	6.0
$\sigma^2_{RV(L)}$	-.0015	.0[2]
σ^2_{LC}	.0631	36.7
σ^2_{CV}	.0007	.5
σ^2_{CVL}	.0172	10.0
Error (σ^2)	.0196	11.4
σ^2_{plot}	.1718	100.0
ΣC_i^2	.1006	-----

[1] σ^2_{plot} = 0.0316 + ... + 0.0196.
[2] Small negative component which was assumed to estimate zero.

where cuttings were not significantly different. As an average of eight locations, the first cutting was low and the second and third cuttings were approximately equal in saponin content.

The eight locations fall into three classes for saponin content with Utah low; Iowa, Kansas irrigated, New York, and Pennsylvania intermediate; and Kansas dryland, Nebraska, and North Carolina high. This relationship was not consistent for all cuttings. However, North Carolina was one of the three locations having the highest saponin content for each cutting.

Varieties and Cuttings Not Common to All Locations

Variety, cutting, and cutting x variety means for all characters measured are given for each of the 10 locations in appendix, tables 9 to 18. Du Puits, which was included only in the two Kansas tests and in Utah, was highest in percentage of saponin at each of these three locations. African, included only in North Carolina and California, had the lowest saponin content in both tests. Caliverde, evaluated only in the California test, was highest at this location.

TABLE 6.--Mean saponin content of alfalfa for cuttings, varieties, and locations

[Expressed as percentage of dry matter]

Varieties	Kans. (dry-land)	Nebr.	N. C.	Pa.	Utah	Iowa	Kans. (irri-gated)	N. Y.	8 loca-tions	Oreg.	Calif.
					First cutting						
Buffalo.....	2.37	2.41	2.97	2.30	2.43	2.16	2.16	2.01	2.35	1.92	2.44
Lahontan.....	1.96	2.02	2.53	2.56	2.18	1.78	2.08	1.96	2.13	1.58	2.18
Ranger.....	2.05	2.46	2.70	2.22	2.30	1.88	2.24	2.13	2.25	1.92	----
Vernal.....	2.37	2.51	3.00	2.16	2.29	2.12	2.65	1.98	2.38	1.88	----
Average.....	2.19	2.35	2.80	2.31	2.30	1.98	2.28	2.02	2.28	1.82	2.31
					Second cutting						
Buffalo.....	3.22	2.71	2.84	2.49	2.09	2.78	2.83	3.01	2.75	----	2.52
Lahontan.....	2.27	2.42	2.34	2.49	1.85	2.82	2.19	2.96	2.42	----	2.46
Ranger.....	3.18	2.76	2.65	2.80	2.17	2.84	2.41	3.27	2.76	----	----
Vernal.....	3.48	2.85	3.00	2.67	2.51	2.95	2.84	3.16	2.93	----	----
Average	3.04	2.68	2.71	2.61	2.16	2.85	2.57	3.10	2.72	----	2.49
					Third cutting						
Buffalo.....	3.13	3.08	2.95	2.72	2.21	2.96	2.60	2.41	2.76	3.23	2.69
Lahontan.....	2.97	2.57	2.45	2.25	2.03	2.56	2.33	2.11	2.41	2.58	2.37
Ranger.....	3.06	3.12	2.58	2.67	2.37	2.74	2.75	2.44	2.72	3.40	----
Vernal.....	2.90	3.59	3.06	2.53	2.43	2.67	3.02	2.71	2.86	3.62	----
Average.....	3.02	3.09	2.76	2.54	2.26	2.73	2.68	2.42	2.69	3.21	2.53
					Seasonal average						
Buffalo.....	2.91	2.73	2.92	2.50	2.24	2.63	2.53	2.48	2.62	2.58	2.55
Lahontan.....	2.40	2.34	2.44	2.43	2.02	2.39	2.20	2.34	2.32	2.08	2.34
Ranger.....	2.76	2.78	2.64	2.56	2.28	2.49	2.47	2.61	2.57	2.66	----
Vernal.....	2.92	2.98	3.02	2.45	2.41	2.58	2.84	2.62	2.73	2.75	----
Average.....	2.75	2.71	2.76	2.48	2.24	2.52	2.51	2.51	2.56	2.52	2.44

Differences in cutting means were significant at all locations except California, North Carolina, Pennsylvania, and Utah. Significant cutting x variety interactions were found for these four locations except North Carolina. Of the locations showing significant differences among cuttings, only the two Kansas locations had significant cutting x variety interactions.

Relationship of Saponin Content to Yield, Defoliation, and Other Chemical Constituents

Association with yield.--Saponin content and yield were significantly negatively correlated for the main effect of cuttings and for the interaction effects of varieties x locations and cuttings x varieties (table 7). A correlation coefficient is regarded significant if the sum of squares due to regression was significant at the 5-percent level when saponin was used either as the "X" or "Y" variable. The first cutting was low in saponin and the other two cuttings were high, while the first cutting was high in yield relative to the other two cuttings, thus accounting for the negative association over cuttings (table 8). Conversely, the correlation for variety means was positive and highly significant, primarily because Lahontan, the lowest yielding variety, was also lowest in saponin.

Except for California, the individual location analyses of variance (appendix, tables 19 to 28) agreed well with the combined analysis. For California, which was not included in the combined analysis, the cuttings correlation was positive and highly significant. Of the other locations, only Ames, Iowa, and Raleigh, N.C., had significant correlations over varieties, while only University Park, Pa., had a significant correlation over cuttings. In these three cases, the sign of the correlation was the same as that obtained from the combined analysis.

Association with defoliation score.--The highly significant negative correlation found for percentage of saponin and defoliation among variety means (table 7) arose because Lahontan, which was consistently low in saponin, was also consistently high in defoliation score (table 8). For locations and cuttings where defoliation score was the same for all varieties--Kansas irrigated, second cut; Nebraska, all cuttings; and Pennsylvania, first two cuttings--Lahontan was lowest in saponin content of the four "core" varieties in all cases except the first cutting in Pennsylvania.

Association with protein.--Only the correlations for cuttings and the interaction cuttings x varieties were significant in the combined analysis (table 7). The significant correlation for cuttings resulted from low saponin and protein contents in the first cutting with an increased level of both saponin and protein in the second and third cuttings. The significant correlation for the interaction of varieties x cuttings indicated a significant association of cutting x variety interaction effects for saponin and protein.

A highly significant negative correlation of saponin and protein found among variety means in Utah (appendix, table 23) resulted from inclusion of Du Puits and Tourneur 501 in the analysis. Du Puits and Tourneur 501 were lower in protein and higher in saponin than the other varieties tested (appendix table 13).

Associations with fiber.--The significant negative correlation among variety means resulted from Lahontan and Ranger being higher in fiber and lower in saponin than the other two varieties (tables 7 and 8). The negative correlation of saponin and fiber for cutting means resulted from the first cut being high in fiber and low in saponin, while the other two cuts were lower in fiber and higher in saponin.

Associations with ash.--The cuttings main effect and the cutting x variety interaction correlations were positive and significant for this character (table 7). The cuttings correlation resulted from low ash and saponin in the first cutting as compared to the other two cuttings (table 8).

Associations with fat.--The correlations between fat and saponin among variety means and among cuttings were highly significant and positive (table 7). Again the fact that Lahontan was low in fat and saponin compared to the other varieties caused the

TABLE 7.--Percentage of reductions in sums of squares due to regression (r^2) on saponin content for certain sources of variation in the analysis of variance of chemical constituents, defoliation score, and yield. (See appendix tables 29 and 30 for mean squares.)

Source of variation	Protein	Crude fiber	Ash	Fat	N-free extract	Defoliation	Hay yield
Locations (L)....	+23.8	+0.2	-3.2	-27.1	-6.2	+24.1	-33.3
Varieties (V)....	+43.9	-88.8 ††	+16.0	+97.8†† **	+8.9	-92.9†† **	+90.1†† **
V x L.....	+3.5	-3.3	+6.5	+12.7	+.3	-3.1	-38.7 †† **
Cuttings (C).....	+81.4 †† **	-100.0 †† **	+91.1††	+93.3 †† *	+76.9 †† *	-10.7	-99.4 †† **
C x L.....	-.3	-23.9	+.8	+4.9	+23.7	-4.3	-2.2
C x V.....	+78.6†† **	-27.8	+64.4† **	+35.8	-2.2	-10.2	-53.9**
C x V x L.....	+1.0	0	-1.1	-.1	0	+10.3	-.4

\+ or - indicates sign of r;
* significant at the 5-percent level with saponin used as the x variable;
** significant at the 1-percent level with saponin used as the x variable;
† significant at the 5-percent level with saponin used as the y variable;
†† significant at the 1-percent level with saponin used as the y variable.

TABLE 8.--Average chemical composition, defoliation score, and hay yield for cuttings and varieties of alfalfa averaged over the eight locations in the "core" of the study

Varieties	Saponin (percent)	Protein (percent)	Crude fiber (percent)	Ash (percent)	Fat (percent)	N-free extract (percent)	Defoliation score[1]	Hay yield per acre (tons)
	First cutting							
Buffalo...	2.35	17.25	33.76	9.00	2.59	37.44	1.80	2.13
Lahontan..	2.13	17.64	34.79	9.14	2.56	35.89	2.40	1.87
Ranger....	2.25	17.54	35.03	8.84	2.58	36.04	2.00	2.24
Vernal....	2.39	17.24	35.73	8.54	2.55	35.99	1.80	2.29
Average.	2.28	17.42	34.83	8.88	2.57	36.34	2.00	2.13
	Second cutting							
Buffalo...	2.75	18.97	28.89	9.51	2.88	39.77	0.80	1.36
Lahontan..	2.42	18.63	29.59	9.04	2.67	40.04	1.40	1.25
Ranger....	2.76	19.46	29.00	9.42	2.79	39.34	.80	1.31
Vernal....	2.93	19.65	28.77	9.30	2.87	39.43	.80	1.35
Average.	2.72	19.18	29.06	9.32	2.80	39.65	.95	1.32
	Third cutting							
Buffalo...	2.76	19.93	29.76	9.56	2.89	37.88	1.80	1.23
Lahontan..	2.41	19.78	30.73	9.22	2.63	37.65	3.00	.91
Ranger....	2.72	20.53	29.66	9.42	2.92	37.48	1.80	1.07
Vernal....	2.86	20.81	27.95	9.66	3.03	38.56	1.70	1.08
Average.	2.69	20.26	29.53	9.45	2.87	37.89	2.08	1.07
	Average of all cuttings							Total of cuttings
Buffalo...	2.62	18.78	30.61	9.37	2.79	38.45	1.40	4.93
Lahontan..	2.33	18.73	31.64	9.14	2.62	37.87	2.22	4.20
Ranger....	2.57	19.24	31.21	9.24	2.78	37.55	1.45	4.81
Vernal....	2.75	19.32	30.56	9.18	2.83	38.14	1.38	4.88
Average.	2.57	19.02	31.01	9.23	2.76	38.00	1.61	4.71

[1] From 0 to 7, representing 0-to-70 percent defoliation, respectively.

significant variety correlation, while low saponin and fat in the first cutting relative to the second and third cuttings caused the significant correlation among cuttings (table 8).

Associations with nitrogen-free extract.--The correlation between saponin and nitrogen-free extract for cutting means was positive and significant, but none of the other correlations between saponin and nitrogen-free extract were significant. The positive correlation resulted from low saponin and nitrogen-free extract in the first cutting compared to the second and third cuttings.

DISCUSSION

The cutting x location interaction accounted for the largest percentage (36.7 percent) of the plot variation for saponin of any of the components included in the analysis. Locations and varieties were the next largest in percentage contributions (18.4 and 16.4 percent, respectively). Although cuttings were treated as a fixed effect and could not be included in the variance component estimate for plots, variation among cuttings was greater than that for any other variable in the experiment. This was not true for every location, however, since cuttings were not significantly different in saponin at three locations. These results indicated that saponin content is under both genetic and environmental control.

The relative importance of the several sources of variation for any particular experiment will be influenced by the varieties, locations, and cuttings included in that experiment. Had Lahontan not been included in this test, the variety component would have been greatly reduced. Conversely, if Du Puits and African had been included in the overall analysis, and their performance had been consistent with that observed in the few locations where each was included, the variety component would have been increased materially. With the variation observed among varieties, it would seem reasonable to conclude that saponin content can be altered by breeding. The extent to which saponin content can be altered by breeding will depend on the genetic variation among individual plants, which was not measured in this study.

If a particular level of saponin were desired in a product derived from alfalfa, judicious selection of the location and cutting from which alfalfa is obtained would aid in getting the desired saponin content. Although years were not measured as a variable in this study, it seems reasonable to assume that saponin content would vary with years, and that years would affect the relative importance of the other variables studied.

The pattern of variation for saponin content was very different from that for coumestrol.[7] In the coumestrol study, the effect of varieties was smaller; environmental effects were larger. This result would seem to indicate that saponin content might be more easily altered by breeding than coumestrol content.

In interpreting the associations between saponin and the other characters at the variety level, it is necessary to consider the characteristics of the varieties involved. Lahontan is lower in yield at the locations studied and is more susceptible than the other varieties to leafspot diseases, which are important in the Eastern United States. Hence, it would be expected to show a higher defoliation score than the other varieties. Since Lahontan had the lowest saponin content of the four varieties in the "core" of the study, a significant positive correlation of saponin with yield and a significant negative correlation with defoliation score resulted. Because stems are higher in crude fiber than leaves, and varieties with a higher defoliation score would have a lower percentage of leaves, a negative correlation of saponin with crude fiber would be expected, and this was obtained. It might be concluded that Lahontan was low in saponin because of

[7] Hanson, C. H., and others. Coumestrol Content of Alfalfa as Related to Location, Cutting, Variety, Stage of Growth, and Other Variables. [In preparation.]

defoliation. However, examination of data from the six cutting-location combinations in which the mean defoliation score was the same for all varieties indicated that in every case except one, Lahontan had the lowest saponin content of the four "core" varieties. Thus, the genetic difference in saponin content between Lahontan and other varieties apparently is not accounted for by differences in defoliation.

The "core" varieties--Lahontan, Buffalo, Ranger, and Vernal--differed only slightly in time of flowering. African and Du Puits flowered somewhat earlier than any of the "core" varieties, but were lower and higher, respectively, in saponin content at locations where either was included. Time of flowering, per se, did not appear to have a consistent effect on saponin content.

Highly significant correlations between saponin and all other characters except defoliation were found for cuttings. The associations over cuttings result from the high yield and crude fiber content of the first cutting and the low protein, ash, fat, and N-free substances of the first cutting compared to the second and third cuttings. The second and third cuttings were similar for all characters for which significant correlations with saponin were noted. High first cutting yields, coupled with high crude fiber and lower protein content, is the usual situation at the locations in the "core" of the study. The difference in yield between first and later cuttings can be accounted for by moisture and other environmental conditions being more favorable for forage production at the time of the first cutting. Since saponin was low in the first cutting, it appears that environmental factors which favor high yield favor low saponin. This is the opposite of the genetic situation, wherein the lowest yielding varieties were also lowest in saponin.

The correlations involving varieties are those of most concern to the plant breeder since these are essentially genetic correlations. If the breeder is interested in lowering saponin content, a decrease in yield and fat content, coupled with an increase in defoliation and fiber content, would be indicated by the correlations obtained. However, as has already been pointed out, low saponin can be obtained without differences in defoliation among varieties. The correlations obtained are among variety means and may or may not represent the genetic correlations which exist among individual plants within varieties. Since selection programs deal with individual plants, it may be possible to develop low saponin varieties with high yield and low fiber.

SUMMARY

Three cuttings of the alfalfa varieties Buffalo, Ranger, Lahontan, and Vernal at Ithaca, N.Y.; University Park, Pa.; Raleigh, N.C.; Ames, Iowa; Lincoln, Nebr.; Manhattan, Kans. (one test each on dryland and irrigated farms); and Logan, Utah, were evaluated for saponin content, protein, crude fiber, ash, fat, nitrogen-free extracts, defoliation score, and hay yield. The four varieties, three cuttings, and eight locations constituted the "core" of the experiment on which the combined statistical analyses were conducted. Information was obtained on additional varieties and cuttings at some locations, on two cuttings of the "core" varieties at Medford, Oreg., and on a different set of varieties at Davis, Calif.

Components of variance making significant contributions to plot-to-plot variation in saponin content and the percentages contributed by each were cuttings x locations, 36.7 percent; locations, 18.4 percent; varieties, 16.4 percent; error, 11.4 percent; cuttings x varieties x locations, 10.0 percent; and locations x varieties, 6.0 percent.

The significant contribution of the variety component indicated that saponin content can be altered by breeding. Since cuttings were assumed to be a fixed variable in the analysis, a component for cuttings was not included in the plot-to-plot variation. However, it was apparent that variation among cuttings was greater than for any other variable.

Of the varieties in the "core" of the experiment, Lahontan was lowest in saponin content. There was little variation among the other varieties. African, included only at Raleigh, N.C., and Davis, Calif., was lower in saponin than Lahontan. Caliverde was higher in saponin than any of the "core" varieties at Davis, Calif., the only location where it was tested. Du Puits, tested only at Logan, Utah, and in the two Kansas tests, was higher in saponin than any of the "core" varieties.

The first cutting averaged lower than the second and third in saponin content. The second and third cuttings were similar.

Over cuttings, saponin was significantly positively correlated with protein, ash, fat, and N-free extract and significantly negatively correlated with crude fiber and hay yield. These correlations resulted primarily from the difference between the first cutting and the other two cuttings for the characters involved. The first cutting was low in saponin and high in yield compared to the other two cuttings.

Among varieties, significant positive correlations of saponin with hay yield and fat content, and significant negative correlations of saponin with crude fiber and defoliation score were found. These correlations resulted primarily from differences between Lahontan and other varieties.

The importance of these correlations in a breeding program aimed at changing saponin content will depend on the extent to which they represent the genetic correlations which exist among individual plants.

LITERATURE CITED

(1) American Association of Cereal Chemists.
1957. Cereal Laboratories Methods. Ed. 6, 528 pp., St. Paul.

(2) Anderson, J. O.
1957. Effect of Alfalfa Saponin on the Performance of Chicks and Laying Hens. Poultry Sci. 36: 873-876.

(3) Coulson, C. B.
1958. Saponins. I. Triterpenoid Saponins from Lucerne and Other Species. Jour. Sci. Food and Agr. 9: 281-287, illus.

(4) Djerassi, Carl, Thomas, D. B., Livingston, A. L., and Thompson, C. R.
1957. Terpenoids. XXXI. The Structure and Stereochemistry of Medicagenic Acid. Jour. Amer. Chem. Soc. 79: 5292-5297.

(5) Heywang, B. W., and Bird, H. R.
1954. The Effect of Alfalfa Saponins on the Growth, Diet Consumption, and Efficiency of Diet Utilization of Chicks. Poultry Sci. 33: 239-241.

(6) Heywang, Thompson, C.R., and Kemmerer, A.R.
1959. Effect of Alfalfa Saponin on Laying Chickens. Poultry Sci. 38: 968-971.

(7) Lindahl, I. L., Shalkop, W. T., Dougherty, R. W., and others.
1957. Alfalfa Saponins. Studies on Their Chemical, Pharmacological, and Physiological Properties in Relation to Ruminant Bloat. U.S. Dept. Agr. Tech. Bul. 1161, 83 pp., illus.

(8) Livingston, A. L.
1959. Lucernic Acid, a New Triterpene from Alfalfa. Jour. Organ. Chem. 24: 1567-1568.

(9) Morris, R. J., Dye, W. B., and Gisler, P. S.
1961. Isolation, Purification, and Structural Identity of an Alfalfa Root Saponin. Jour. Organ. Chem. 26: 1241-1243, illus.

(10) Peterson, D. W.
1950. Some Properties of a Factor in Alfalfa Meal Causing Depression of Growth in Chicks. Jour. Biol. Chem. 183: 647-653.

(11) Potter, G. C., and Kummerow, F. A.
1954. Chemical Similarity and Biological Activity of the Saponins Isolated from Alfalfa and Soybeans. Science 120: 224-225, illus.

(12) Shaw, R. A., and Jackson, H. D.
1959. Isolation of a Respiratory Inhibitor from Alfalfa. Arch. Biochem. and Biophys. 84: 405-410, illus.

(13) Snedecor, G. W.
1956. Statistical Methods. Ed. 5, 534 pp., illus. Ames, Iowa.

(14) Sollman, T. H.
1957. A Manual of Pharmacology and Its Applications to Therapeutics and Toxicology. Ed. 8, 1535 pp. Philadelphia.

(15) Van Atta, G. R., and Guggolz, Jack.
1958. Forage Constituents; Detection of Saponins and Sapogenins on Paper Chromatograms by Liebermann-Burchard Reagent. Jour. Agr. and Food Chem. 6: 849-850, illus.

(16) Van Atta, G. R., Guggolz, Jack, and Thompson, C. R.
1961. Determination of Saponins in Alfalfa. Jour. Agr. and Food Chem. 9: 77-79, illus.

(17) Walter, E. D., Bickoff, E. M., Thompson, C. R., Robinson, C. H., and Djerassi, Carl.
1955. Saponin from Ladino Clover (Trifolium repens). Jour. Amer. Chem. Soc. 77: 4936-4937.

(18) _____ Van Atta, G. R., Thompson, C. R. and Maclay, W.D.
1954. Alfalfa Saponin. Jour. Amer. Chem. Soc. 76: 2271-2273.

TABLE 9.--Chemical composition, defoliation score, and hay yield for varieties and cuttings of alfalfa at Manhattan, Kans. (dryland)

Variety	Saponin (percent)	Protein (percent	Crude fiber (percent)	Ash (percent)	Fat (percent)	N-free extract (percent)	Defoliation 1/ score	Hay yield per acre (tons)
				First cutting				
Buffalo----	2.37	17.78	37.41	8.61	2.34	33.88	3.00	1.88
Lahontan---	1.96	17.32	36.81	8.46	2.31	35.12	4.00	1.78
Ranger-----	2.05	16.92	38.44	8.08	2.54	34.03	3.00	2.14
Vernal-----	2.37	17.85	37.59	8.10	2.25	34.23	3.00	2.03
Du Puits---	2.64	15.67	37.93	8.69	1.95	35.78	5.00	2.26
Average--	2.28	17.11	37.64	8.39	2.28	34.61	3.60	2.02
				Second cutting				
Buffalo----	3.22	19.43	25.75	9.49	2.25	43.09	0	1.23
Lahontan---	2.27	19.23	27.92	9.24	2.16	41.46	0	1.19
Ranger-----	3.18	20.07	25.83	9.40	2.23	42.47	0	1.29
Vernal-----	3.48	21.28	24.90	9.42	2.35	42.06	0	1.09
Du Puits---	3.88	19.20	27.63	8.90	2.29	41.98	0	1.30
Average--	3.21	19.84	26.41	9.29	2.26	42.21	0	1.22
				Third cutting				
Buffalo----	3.13	18.92	27.36	9.73	3.28	40.72	2.00	.86
Lahontan---	2.97	18.74	29.70	8.98	3.03	39.56	4.00	.81
Ranger-----	3.06	19.53	26.94	10.19	3.20	40.15	2.00	.83
Vernal-----	2.90	19.21	24.88	10.15	3.51	42.26	2.00	.62
Du Puits---	3.80	18.75	26.01	9.52	3.05	42.68	2.00	.71
Average--	3.17	19.03	26.98	9.71	3.21	41.07	2.40	.77
				Average of all cuttings				Total of cuttings
Buffalo----	2.91	18.71	30.17	9.28	2.62	39.23	1.67	3.97
Lahontan---	2.40	18.43	31.48	8.89	2.50	38.71	2.67	3.78
Ranger-----	2.76	18.84	30.40	9.22	2.66	38.88	1.67	4.26
Vernal-----	2.92	19.45	29.12	9.22	2.70	39.52	1.67	3.74
Du Puits---	3.44	17.87	30.52	9.04	2.43	40.15	2.33	4.27
Average--	2.89	18.66	30.34	9.13	2.58	39.30	2.00	4.00

1/ From 0 to 7, representing 0- to 70-percent defoliation, respectively.

TABLE 10.--Chemical composition, defoliation score, and hay yield for varieties and cuttings of alfalfa at Lincoln, Nebr.

Variety	Saponin (percent)	Protein (percent)	Crude fiber (percent)	Ash (percent)	Fat (percent)	N-free extract (percent)	Defoliation score 1/	Hay yield per acre (tons)
				First cutting				
Buffalo----	2.41	17.66	32.36	9.91	2.41	37.77	1.00	2.67
Lahontan---	2.02	17.23	38.58	9.61	2.39	32.20	1.00	2.25
Ranger-----	2.46	17.31	36.16	9.57	2.39	34.59	1.00	2.61
Vernal-----	2.51	17.36	37.83	9.26	2.40	33.45	1.00	2.61
Average--	2.35	17.39	36.21	9.59	2.40	34.50	1.00	2.54
				Second cutting				
Buffalo----	2.71	21.16	29.73	11.31	2.19	35.63	1.00	1.54
Lahontan---	2.42	20.03	32.72	10.83	2.25	34.18	1.00	1.41
Ranger-----	2.76	21.43	29.23	11.29	2.14	35.92	1.00	1.59
Vernal-----	2.85	21.21	28.64	10.78	2.30	37.09	1.00	1.40
Average--	2.68	20.96	30.08	11.05	2.22	35.71	1.00	1.49
				Third cutting				
Buffalo----	3.08	19.29	29.44	10.15	2.74	38.38	1.00	1.51
Lahontan---	2.57	18.59	33.68	9.61	2.48	35.66	1.00	1.44
Ranger-----	3.12	19.00	32.43	9.82	2.34	36.42	1.00	1.49
Vernal-----	3.59	19.70	29.89	10.20	2.84	37.39	1.00	1.39
Average--	3.09	19.15	31.36	9.95	2.60	36.96	1.00	1.46
				Average of all cuttings				Total of cuttings
Buffalo----	2.73	19.37	30.48	10.46	2.45	37.26	1.00	5.72
Lahontan---	2.34	18.62	34.99	10.02	2.37	34.01	1.00	5.10
Ranger-----	2.78	19.25	32.61	10.23	2.29	35.64	1.00	5.69
Vernal-----	2.98	19.42	32.12	10.08	2.51	35.98	1.00	5.40
Average--	2.71	19.16	32.55	10.20	2.40	35.72	1.00	5.48

1/ From 0 to 7, representing 0- to 70-percent defoliation, respectively.

TABLE 11.--Chemical composition and hay yield for varieties and cuttings of alfalfa at Raleigh, N. C.

Variety	Saponin (percent)	Protein (percent)	Crude fiber (percent)	Ash (percent)	Fat (percent)	N-free extract (percent)	Hay yield per acre (tons)
			First cutting				
Buffalo----	2.97	22.68	27.01	9.99	3.37	36.96	1.26
Lahontan---	2.53	21.43	29.90	9.44	3.32	35.91	1.26
Ranger-----	2.70	22.84	29.05	10.15	3.43	34.55	1.32
Vernal-----	3.00	21.87	33.27	9.06	3.31	32.50	1.60
African----	1.93	22.25	26.40	10.22	3.68	37.47	.86
Average--	2.63	22.21	29.13	9.77	3.42	35.48	1.26
			Second cutting				
Buffalo----	2.84	22.23	25.92	9.32	3.02	39.53	.85
Lahontan---	2.34	22.58	25.53	9.43	2.48	39.99	.90
Ranger-----	2.65	24.40	26.16	9.32	2.49	37.64	.72
Vernal-----	3.00	23.94	26.86	9.07	2.85	37.30	.93
African----	2.25	21.20	27.10	9.01	3.54	39.16	.83
Average--	2.62	22.87	26.31	9.23	2.88	38.72	.85
			Third cutting				
Buffalo----	2.95	22.19	31.63	8.78	2.73	34.68	1.03
Lahontan---	2.45	22.24	30.16	9.02	2.57	36.02	.74
Ranger-----	2.58	23.48	33.11	8.29	2.70	32.44	.96
Vernal-----	3.06	24.64	29.67	8.79	3.02	33.89	.91
African----	2.31	22.16	28.86	9.12	3.08	36.79	.70
Average--	2.67	22.94	30.69	8.80	2.82	34.76	.87
			Average of all cuttings				Total of cuttings
Buffalo----	2.92	22.37	28.19	9.36	3.04	37.06	3.14
Lahontan---	2.44	22.08	28.53	9.30	2.79	37.30	2.90
Ranger-----	2.64	23.57	29.44	9.25	2.87	34.88	3.00
Vernal-----	3.02	23.48	29.93	8.97	3.06	34.56	3.44
African----	2.16	21.87	27.45	9.45	3.43	37.81	2.39
Average--	2.64	22.67	28.71	9.27	3.04	36.32	2.97

TABLE 12.--Chemical composition, defoliation score, and hay yield for varieties and cuttings of alfalfa at University Park, Pa.

Variety	Saponin (percent)	Protein (percent)	Crude fiber (percent)	Ash (percent)	Fat (percent)	N-free extract (percent)	Defoliation score 1/	Hay yield per acre (tons)
				First cutting				
Buffalo------	2.30	15.53	35.25	8.33	2.64	38.26	1.00	1.99
Lahontan-----	2.56	16.46	32.92	8.67	2.78	39.18	1.00	1.57
Ranger-------	2.22	16.48	35.56	8.73	2.57	36.67	1.00	2.34
Vernal-------	2.16	16.88	36.20	8.37	2.20	36.36	1.00	2.25
Average----	2.31	16.34	34.98	8.53	2.55	37.62	1.00	2.04
				Second cutting				
Buffalo------	2.49	18.85	27.26	8.51	3.87	41.52	2.00	1.06
Lahontan-----	2.49	19.03	26.86	8.28	3.54	42.30	2.00	.78
Ranger-------	2.80	20.43	32.04	8.68	3.82	35.04	2.00	1.01
Vernal-------	2.67	19.39	32.69	8.33	3.32	36.29	2.00	1.45
Average----	2.61	19.43	29.71	8.45	3.64	38.79	2.00	1.08
				Third cutting				
Buffalo------	2.72	20.94	30.75	8.97	3.28	36.07	1.00	1.32
Lahontan-----	2.25	20.99	32.42	8.55	2.70	35.36	2.00	.94
Ranger-------	2.67	21.43	31.87	8.92	3.20	34.60	1.00	1.17
Vernal-------	2.53	20.89	29.48	8.92	3.25	37.47	1.00	1.36
Average----	2.54	21.06	31.13	8.84	3.11	35.88	1.25	1.20
				Average of all cuttings				Total of cuttings
Buffalo------	2.50	18.44	31.09	8.60	3.26	38.62	1.33	4.37
Lahontan-----	2.43	18.83	30.73	8.50	3.01	38.95	1.67	3.29
Ranger-------	2.56	19.45	33.16	8.78	3.20	35.44	1.33	4.52
Vernal-------	2.45	19.05	32.79	8.54	2.92	36.71	1.33	5.06
Average----	2.48	18.94	31.94	8.60	3.10	37.43	1.42	4.31

1/ From 0 to 7, representing 0- to 70-percent defoliation, respectively.

TABLE 13.--Chemical composition and hay yield for varieties and cuttings of alfalfa at Logan, Utah

Variety	Saponin (percent)	Protein (percent)	Crude fiber (percent)	Ash (percent)	Fat (percent)	N-free extract (percent)	Hay yield per acre (tons)
			First cutting				
Buffalo--------	2.43	17.92	31.24	10.17	3.21	37.47	3.02
Lahontan-------	2.18	18.10	31.05	10.64	2.91	37.32	2.47
Ranger---------	2.30	16.98	31.00	9.86	3.25	38.92	3.04
Vernal---------	2.29	17.15	31.30	10.16	3.38	38.02	3.18
Du Puits-------	2.85	17.40	32.96	10.60	3.27	35.78	3.18
Tourneur 501---	2.68	16.45	32.31	9.98	3.44	37.83	3.09
Average------	2.46	17.33	31.64	10.24	3.24	37.56	3.00
			Second cutting				
Buffalo--------	2.09	17.80	30.60	10.29	3.45	37.88	1.76
Lahontan-------	1.85	17.05	32.49	9.44	3.45	37.58	1.73
Ranger---------	2.17	17.72	29.52	10.44	3.17	39.16	1.40
Vernal---------	2.51	18.41	26.77	10.54	3.73	40.56	1.58
Du Puits-------	2.63	16.26	32.03	10.00	3.39	38.43	1.73
Tourneur 501---	2.74	17.20	29.86	10.78	3.18	39.00	1.69
Average------	2.33	17.41	30.21	10.25	3.40	38.77	1.65
			Third cutting				
Buffalo--------	2.21	20.59	26.82	11.26	3.34	38.00	1.73
Lahontan-------	2.03	20.29	27.66	11.88	2.75	37.43	.95
Ranger---------	2.37	21.35	24.77	11.47	3.56	38.86	1.12
Vernal---------	2.43	21.44	25.33	11.76	2.95	38.54	1.37
Du Puits-------	3.01	19.10	28.44	11.14	3.36	37.98	1.14
Tourneur 501---	3.02	19.55	31.89	11.23	3.38	33.96	1.25
Average------	2.51	20.39	27.49	11.46	3.22	37.46	1.26
			Average of all cuttings				Total of cuttings
Buffalo--------	2.24	18.77	29.55	10.57	3.33	37.78	6.51
Lahontan-------	2.02	18.48	30.40	10.65	3.04	37.44	5.15
Ranger---------	2.28	18.68	28.43	10.59	3.33	38.98	5.56
Vernal---------	2.41	19.00	27.80	10.82	3.35	39.04	6.13
Du Puits-------	2.83	17.59	31.14	10.58	3.34	37.40	6.05
Tourneur 501---	2.81	17.73	31.35	10.66	3.33	36.93	6.03
Average------	2.43	18.38	29.78	10.64	3.29	37.93	5.90

TABLE 14.--Chemical composition, defoliation score, and hay yield for varieties and cuttings of alfalfa at Ames, Iowa

Variety	Saponin (percent)	Protein (percent)	Crude fiber (percent)	Ash (percent)	Fat (percent)	N-free extract (percent)	Defoliation score 1/	Hay yield per acre (tons)
				First cutting				
Buffalo----	2.16	13.64	36.40	7.42	1.91	40.63	1.00	2.17
Lahontan---	1.78	16.00	36.20	8.03	2.13	37.66	2.00	1.68
Ranger-----	1.88	16.16	37.97	6.99	1.93	36.97	1.00	2.34
Vernal-----	2.12	13.72	34.58	6.49	2.03	43.19	1.00	2.29
Average--	1.98	14.88	36.29	7.23	2.00	39.61	1.25	2.12
				Second cutting				
Buffalo----	2.78	16.48	29.19	8.54	2.97	42.83	1.00	.93
Lahontan---	2.82	16.25	30.69	7.39	2.53	43.15	2.00	.70
Ranger-----	2.84	16.27	29.21	7.87	3.17	43.49	1.00	.95
Vernal-----	2.95	17.56	26.48	8.35	2.81	44.81	1.00	.91
Average--	2.85	16.64	28.89	8.04	2.87	43.57	1.25	.87
				Third cutting				
Buffalo----	2.96	20.25	32.70	8.89	2.34	35.84	1.00	.85
Lahontan---	2.56	18.73	30.61	7.64	2.36	40.67	2.00	.45
Ranger-----	2.74	20.24	28.92	7.86	2.31	40.67	1.00	.69
Vernal-----	2.67	21.06	27.98	8.37	2.60	40.01	1.00	.74
Average--	2.73	20.07	30.05	8.19	2.40	39.30	1.25	.68
				Average of all cuttings				Total of cuttings
Buffalo----	2.63	16.79	32.76	8.28	2.41	39.77	1.00	3.95
Lahontan---	2.39	16.99	32.50	7.69	2.34	40.49	2.00	2.83
Ranger-----	2.49	17.56	32.03	7.57	2.47	40.38	1.00	3.98
Vernal-----	2.58	17.45	29.68	7.74	2.48	42.67	1.00	3.94
Average--	2.52	17.20	31.74	7.82	2.42	40.83	1.25	3.68

1/ From 0 to 7, representing 0- to 70-percent defoliation, respectively.

TABLE 15.--Chemical composition, defoliation score, and hay yield for varieties and cuttings of alfalfa at Manhattan, Kans. (irrigated)

Variety	Saponin (percent)	Protein (percent)	Crude fiber (percent)	Ash (percent)	Fat (percent)	N-free extract (percent)	Defoliation score 1/	Hay yield per acre (tons)
	First cutting							
Buffalo----	2.16	18.00	36.87	10.51	2.58	32.05	3.00	1.95
Lahontan---	2.08	18.04	37.81	10.06	2.41	31.69	4.00	2.06
Ranger-----	2.24	18.11	38.23	9.79	2.24	31.64	4.00	1.87
Vernal-----	2.65	18.06	40.39	9.70	2.33	29.53	3.00	2.04
Du Puits---	2.78	16.73	37.95	9.98	1.84	33.51	2.00	2.59
Average--	2.38	17.79	38.25	10.01	2.28	31.68	3.20	2.10
	Second cutting							
Buffalo----	2.83	17.45	34.83	10.68	2.96	34.09	0	2.18
Lahontan---	2.19	16.71	33.97	9.93	2.54	36.55	2.00	2.04
Ranger-----	2.41	17.68	32.36	10.54	2.94	36.49	0	2.22
Vernal-----	2.84	17.48	32.59	10.24	3.10	36.61	0	2.06
Du Puits---	2.95	17.17	32.87	10.38	3.22	36.37	0	2.34
Average--	2.64	17.30	33.32	10.35	2.95	36.02	.40	2.17
	Third cutting							
Buffalo----	2.60	16.73	32.34	9.62	2.65	38.66	4.00	1.30
Lahontan---	2.33	16.65	34.26	9.15	2.50	37.45	6.00	1.03
Ranger-----	2.75	17.18	32.12	9.76	2.66	38.29	4.00	1.21
Vernal-----	3.02	17.39	30.97	10.05	2.83	38.77	3.50	1.14
Du Puits---	3.67	17.15	29.52	9.91	2.52	40.91	4.00	1.08
Average--	2.87	17.02	31.84	9.70	2.63	38.82	4.30	1.15
	Fourth cutting							
Buffalo----	2.74	19.66	28.70	10.96	2.98	37.71	1.00	1.39
Lahontan---	2.60	18.45	33.17	10.07	2.64	35.68	3.00	1.21
Ranger-----	2.19	19.88	33.50	10.45	3.01	33.18	1.00	1.36
Vernal-----	3.54	20.57	26.28	10.47	3.20	39.50	1.00	1.24
Du Puits---	3.10	20.05	25.60	11.29	3.29	39.78	1.00	1.07
Average--	2.83	19.72	29.45	10.65	3.02	37.17	1.40	1.25
	Average of all cuttings							Total of cuttings
Buffalo----	2.58	17.96	33.18	10.44	2.79	35.62	2.00	6.82
Lahontan---	2.30	17.46	34.80	9.80	2.52	35.34	3.75	6.34
Ranger-----	2.40	18.21	34.05	10.14	2.71	34.90	2.25	6.66
Vernal-----	3.01	18.38	32.56	10.12	2.86	36.10	1.88	6.48
Du Puits---	3.12	17.78	31.48	10.39	2.72	37.64	1.75	7.08
Average--	2.68	17.96	33.21	10.18	2.72	35.92	2.33	6.68

1/ From 0 to 7, representing 0- to 70-percent defoliation, respectively.

TABLE 16.--Chemical composition for varieties and cuttings of alfalfa at Ithaca, N. Y.

Variety	Saponin (percent)	Protein (percent)	Crude fiber (percent)	Ash (percent)	Fat (percent)	N-free extract (percent)
			First cutting			
Buffalo----------	2.01	14.76	33.54	7.02	2.22	42.47
Lahontan---------	1.96	16.52	35.08	8.19	2.20	38.02
Ranger-----------	2.13	15.48	33.80	7.56	2.25	40.92
Vernal-----------	1.98	15.00	34.69	7.18	2.47	40.67
Average--------	2.02	15.44	34.28	7.49	2.29	40.52
			Second cutting			
Buffalo----------	3.01	18.32	27.87	7.90	2.36	43.56
Lahontan---------	2.96	18.17	26.52	7.79	2.39	45.14
Ranger-----------	3.27	17.64	27.70	7.82	2.38	44.48
Vernal-----------	3.16	17.91	31.25	7.69	2.46	40.70
Average--------	3.10	18.01	28.34	7.80	2.40	43.47
			Third cutting			
Buffalo----------	2.41	20.50	27.05	9.04	2.75	40.68
Lahontan---------	2.11	22.03	27.35	8.92	2.66	39.06
Ranger-----------	2.44	22.04	27.10	9.07	3.38	38.42
Vernal-----------	2.71	22.18	25.42	9.05	3.23	40.12
Average--------	2.42	21.69	26.73	9.02	3.01	39.57
			Average of all cuttings			
Buffalo----------	2.48	17.86	29.49	7.99	2.44	42.24
Lahontan---------	2.34	18.91	29.65	8.30	2.42	40.74
Ranger-----------	2.61	18.39	29.53	8.15	2.67	41.27
Vernal-----------	2.61	18.36	30.45	7.97	2.72	40.50
Average--------	2.51	18.38	29.78	8.10	2.56	41.19

TABLE 17.--Chemical composition and defoliation score for varieties and cuttings of alfalfa at Medford, Oreg.

Variety	Saponin (percent)	Protein (percent)	Crude fiber (percent)	Ash (percent)	Fat (percent)	N-free extract (percent)	Defoliation score 1/
			First cutting				
Buffalo----	1.92	18.22	28.94	10.80	2.24	39.81	1.00
Lahontan---	1.58	17.40	32.61	10.98	2.07	36.95	0
Ranger-----	1.92	17.72	30.77	10.00	1.97	39.55	1.00
Vernal-----	1.88	18.12	31.06	11.25	2.05	37.53	1.00
Average--	1.82	17.87	30.85	10.76	2.08	38.46	.75
			Third cutting				
Buffalo----	3.23	19.61	26.68	10.39	2.50	40.82	1.00
Lahontan---	2.58	20.46	25.76	10.27	2.66	40.86	1.00
Ranger-----	3.40	20.25	24.48	10.59	2.90	41.79	1.00
Vernal-----	3.62	20.95	20.97	10.09	2.86	45.15	1.00
Average--	3.21	20.32	24.47	10.34	2.73	42.16	1.00
			Average of two cuttings				
Buffalo----	2.58	18.92	27.81	10.60	2.37	40.32	1.00
Lahontan---	2.08	18.93	29.18	10.62	2.36	38.90	.50
Ranger-----	2.66	18.99	27.63	10.30	2.44	40.67	1.00
Vernal-----	2.75	19.54	26.02	10.67	2.46	41.34	1.00
Average--	2.52	19.10	27.66	10.55	2.41	40.31	.88

1/ From 0 to 7, representing 0- to 70-percent defoliation, respectively.

TABLE 18.--Chemical composition and hay yield for varieties and cuttings of alfalfa at Davis, Calif.

Variety	Saponin (percent)	Protein (percent)	Crude fiber (percent)	Ash (percent)	Fat (percent)	N-free extract (percent)	Hay yield per acre (tons)
			First cutting				
Buffalo	2.44	21.90	26.30	11.90	2.49	37.43	1.54
Lahontan	2.18	20.76	27.46	11.85	2.43	37.52	1.64
African	2.29	20.71	28.14	11.05	2.49	37.62	1.90
Caliverde	3.20	21.27	26.24	11.78	2.81	36.66	1.75
Average	2.53	21.16	27.04	11.65	2.56	37.31	1.71
			Second cutting				
Buffalo	2.52	20.31	31.47	10.80	3.14	34.30	1.80
Lahontan	2.46	18.36	28.97	10.90	2.27	39.51	1.89
African	2.18	19.37	29.47	10.98	2.73	37.46	1.84
Caliverde	3.15	19.44	24.42	10.75	2.16	43.25	1.97
Average	2.58	19.37	28.58	10.86	2.58	38.63	1.88
			Third cutting				
Buffalo	2.69	22.26	25.94	10.93	2.94	37.95	1.61
Lahontan	2.37	20.72	28.92	10.82	2.50	37.05	1.83
African	1.97	18.33	32.36	10.37	2.73	36.28	2.02
Caliverde	3.32	21.44	25.41	11.19	2.26	39.70	1.72
Average	2.59	20.69	28.16	10.83	2.61	37.75	1.80
			Fourth cutting				
Buffalo	2.78	22.86	26.98	10.85	2.87	36.46	1.51
Lahontan	2.10	20.20	25.02	10.83	2.33	41.63	1.77
African	2.10	20.58	25.42	11.12	3.20	39.69	1.72
Caliverde	2.82	21.30	25.82	11.52	2.29	39.08	1.38
Average	2.45	21.24	25.81	11.08	2.67	39.22	1.60
			Fifth cutting				
Buffalo	2.41	18.91	27.25	11.23	2.55	40.07	1.24
Lahontan	2.05	19.33	27.11	11.30	2.93	39.34	1.30
African	2.32	17.60	26.71	10.66	2.41	42.63	1.35
Caliverde	2.67	20.13	28.35	11.58	3.00	36.95	1.39
Average	2.36	18.99	27.36	11.19	2.72	39.75	1.32
			Average of all cuttings				Total of cuttings
Buffalo	2.57	21.25	27.59	11.14	2.80	37.24	7.70
Lahontan	2.23	19.87	27.50	11.14	2.49	39.01	8.43
African	2.17	19.32	28.42	10.84	2.71	38.74	8.83
Caliverde	3.03	20.72	26.05	11.36	2.50	39.13	8.21
Average	2.50	20.29	27.39	11.12	2.62	38.53	8.29

TABLE 19.--Mean squares from the analysis of variance of characters analyzed and regressions of saponin ("x" variable" with the other characters, Agronomy Farm, Manhattan, Kans. (dryland) 1/

Source	d.f.	Saponin	Protein	Crude fiber	Ash	Fat	N-free extract	Defoli-ation	Hay yield
Total----------	29	0.3503	1.9584	29.6868	0.4559	0.2481	13.3645	2.1437	0.2965
Replications (R)-	1	.0003	.7083	.3371	.1153	.0022	2.9390	.0334	.0004
Varieties (V)----	4	.8318**	1.9892	4.2509	.1567	.0781	1.9275	1.3333**	.0425
Regression----	1	--------	1.1048-	2.6548-	.0363+	.0269-	7.0445+ **	3.5739-	.0573+
Deviations----	3	--------	2.2839	4.7829*	.1968	.0952	.2218	.5865**	.0376
R x V----------	4	.0118	.3693	.7047	.0567	.0355	1.0694	.0332	.0153
Cuts (C)---------	2	2.7629**	19.7373**	399.7240**	4.5994**	2.9965**	168.1906**	26.4334**	4.0049**
Regression----	1	--------	36.7971+ **	799.2712- **	8.1223+ **	1.2619+	332.4089+ **	25.6732-	6.8154-
Deviations----	1	--------	2.6775	.1768	1.0764	4.7311**	3.9723	27.1935**	1.1944*
C x V----------	8	.1371**	.6778*	3.1638	.3071**	.0499	1.8817	.4333**	.0331*
Regression----	1	--------	2.6350+ **	.0137+	.4485-	.0074-	.9691-	2.5043+ **	.0140-
Deviations----	7	--------	.3982	3.6139	.2869*	.0560	2.0121	.1375*	.0359*
Error b----------	10	.0162	.1754	1.5999	.0598	.0346	2.1209	.0334	.0092

1/ * Significant at the 5-percent level; ** significant at the 1-percent level; + or - following the regression mean squares indicates the sign of the regression coefficient.

TABLE 20.--Mean squares from the analysis of variance of characters analyzed and regressions of saponin ("x" variable) with the other characters, Lincoln, Nebr. 1/

Source	d.f.	Saponin	Protein	Crude fiber	Ash	Fat	N-free extract	Defoliation 2/	Hay yield
Total----------	23	0.1729	0.2809	12.2341	0.4842	0.0508	4.3679	-------	0.2934
Replications (R)-	1	.0631	.0704	3.3825	.0198	.0043	5.9004	-------	.1040
Varieties (V)----	3	.4415**	.8299	20.8971*	.2293	.0560	10.7132	-------	.0561
Regression----	1	--------	2.2254+	31.5578-	.0524+	.0308+	14.9364+	-------	.0600+
Deviations----	2	--------	.1322	15.5668*	.3177	.0686	8.6016	-------	.0542
R x V----------	3	.0063	.5195	1.4760	.0733	.0226	2.0552	-------	.0264
Cuts (C)---------	2	1.0983**	25.4207**	83.5526**	4.6443**	.2873**	12.0928*	-------	3.0118**
Regression----	1	--------	10.0873+	85.2226-	.3088+	.1899+	24.1453+	** -------	4.3456-
Deviations----	1	--------	40.7540**	81.8825**	8.9798**	.3847**	.0404	-------	1.6779**
C x V----------	6	.0381	.2330	4.9118	.1158*	.0302	3.1647	-------	.0199
Regression----	1	--------	.1331+	3.2181-	.4477+ **	.0886+	.1041+	-------	.0138-
Deviations----	5	--------	.2530	5.2505	.0494	.0186	3.7768	-------	.0211
Error b----------	8	.0181	.1116	1.7884	.0283	.0215	1.6353	-------	.0317

1/ * Significant at the 5-percent level; ** significant at the 1-percent level; + or - following the regression mean squares indicates the sign of the regression coefficient.

2/ All varieties scored 1.0.

TABLE 21.--Mean squares from the analysis of variance of characters analyzed and regressions of saponin ("x" variable) with the other characters, Raleigh, N. C. 1/

Source	d.f.	Saponin	Protein	Crude fiber	Ash	Fat	N-free extract	Defoliation 2/	Hay yield
Total----------	29	0.1265	1.5204	8.6598	0.5124	0.2025	7.6598	-----	0.0703
Replications (R)-	1	.0720	2.1547	6.2837	.0864	.0258	12.3905	-----	.1141
Varieties (V)-----	4	.7431**	3.8324	5.8750	.1963	.3646	13.3569	-----	.0989*
Regression-----	1	--------	6.4216+	11.2069+	.3919-	.2062-	23.0549-	-----	.3643+ **
Deviations----	3	--------	2.9693	4.0977	.1310	.4174	10.1242	-----	.0104
R x V----------	4	.0312	1.5224	6.7269	.5519	.1872	6.6325	-----	.0091
Cuts (C)---------	2	.0081	1.6080	49.0288**	2.3681*	1.1094**	44.5946**	-----	.5466**
Regression----	1	--------	.6286+	73.0926+	2.5269- *	.4116-	50.5456-	-----	.1075-
Deviations----	1	--------	2.5873	24.9649*	2.2093	1.8071**	38.6435**	-----	.9856**
C x V----------	8	.0282	1.3209	7.7645	.2899	.0790	1.7191	-----	.0472**
Regression----	1	--------	.6907-	1.0797+	.0461-	.0691+	.0651-	-----	.0542+
Deviations----	7	--------	1.4109	8.7194	.3247	.0805	1.9554	-----	.0462**
Error b----------	10	.0257	.6734	3.4271	.4726	.0789	2.6845	-----	.0023

1/ * Significant at the 5-percent level; ** significant at the 1-percent level; + or - following the regression mean squares indicates the sign of the regression coefficient.

2/ No data available.

TABLE 22.--Mean squares from the analysis of variance of characters analyzed and regressions of saponin ("x" variable) with the other characters, University Park, Pa. 1/

Source	d.f.	Saponin	Protein	Crude fiber	Ash	Fat	N-free extract	Defoliation	Hay yield
Total----------	23	0.0469	0.2396	10.0265	0.0844	0.3354	7.1324	0.2536	0.2663
Replications (R)-	1	.0005	1.2330	4.8780	.1066	.0000	2.0126	.0000	.1162
Varieties (V)----	3	.0202	1.0592	8.7715	.0899	.1520	16.3691	.1667	.3676
Regression----	1	--------	.6863+	8.5048+	.2628+	.2525+	22.6484-	.2085-	.1638+
Deviations----	2	--------	1.2457	8.9049	.0034	.1032	13.2295	.1458	.4695*
R x V----------	3	.0139	.5508	2.1916	.0628	.1619	3.0976	.0000	.0429
Cuts (C)---------	2	.2006	46.0317**	59.5433**	.3442**	2.3821**	17.1994	2.1667**	2.2021**
Regression----	1	--------	65.1844+	118.9030- **	.0092+	4.3799+ **	.4123+	3.0253+	4.3548- **
Deviations----	1	--------	26.8790**	.1835	.6791**	.3842	33.9864	1.3080*	.0493
C x V----------	6	.0882**	.3563	8.8228	.0691	.1259	8.4232*	.1667	.0498*
Regression----	1	--------	.2896+	.2274-	.1668+	.3110+	1.0531-	.4263-	.0530-
Deviations----	5	--------	.3697	10.5418*	.0496	.0888	9.8972*	.1147	.0491*
Error b----------	8	.0057	.5508	2.6024	.0343	.1563	2.3369	.0000	.0092

1/ * Significant at the 5-percent level; ** significant at the 1-percent level; + or - following the regression mean squares indicates the sign of the regression coefficient.

TABLE 23.--Mean squares from the analysis of variance of characters analyzed and regressions of saponin ("x" variable) with the other characters, Logan, Utah 1/

Source	d.f.	Saponin	Protein	Crude fiber	Ash	Fat	N-free extract	Defoliation 2/	Hay yield
Total----------	35	0.1146	2.7642	9.4282	0.5098	0.0699	5.3950	-----	0.7248
Replications (R)-	1	.0529	.5575	2.2801	.0267	.0024	4.1820	-----	2.8449
Varieties (V)----	5	.6469**	2.0298*	12.6065	.0520	.0914	4.6888	-----	.1528
Regression----	1	--------	6.1367- **	15.7542+	.0003-	.1748+	3.5864-	-----	.1507+
Deviations----	4	--------	1.0031	11.8196	.0650	.0706	4.9644	-----	.1533
R x V----------	5	.0049	.3665	5.8930	.1394	.0463	6.3684	-----	.0444
Cuts (C)---------	2	.1011	36.4366**	53.5566**	5.9136**	.1043	6.2198	-----	9.9806**
Regression----	1	--------	38.0321+	23.0507-	6.3027+	.2008-	11.7518-	-----	.0002+
Deviations----	1	--------	34.8411**	84.0624**	5.5244**	.0077	.6878	-----	19.9610**
C x V----------	10	.0399*	.8006*	5.1251	.3954*	.1254**	3.0685	-----	.0850
Regression----	1	--------	1.4007+ *	2.0732-	.2715+	.0086+	.1269-	-----	.3691- *
Deviations----	9	--------	.7340	5.4642	.4092**	.1384**	3.3953	-----	.0534
Error b----------	12	.0083	.2773	6.4038	.0897	.0243	7.1862	-----	.0606

1/ * Significant at the 5-percent level; ** significant at the 1-percent level; + or - following the regression mean squares indicates the sign of the regression coefficient.

2/ No data available.

TABLE 24.--Mean squares from the analysis of variance of characters analyzed and regressions of saponin ("x" variable) with the other characters, Ames, Iowa 1/

Source	d.f.	Saponin	Protein	Crude fiber	Ash	Fat	N-free extract	Defoliation	Hay yield
Total----------	23	0.1879	5.9347	15.9021	0.5718	0.1689	9.5491	0.1957	0.4763
Replications (R)-	1	.0266	.0571	2.7540	.7039	.0000	1.1008	.0000	.2341
Varieties (V)----	3	.0703	.7994	11.9037	.6011	.0253	9.6549	1.5000	.2100*
Regression----	1	--------	.0373-	2.6093-	.8968+	.0207+	.5086+	3.1095-	.4147+ *
Deviations----	2	--------	1.1804*	16.5510	.4532	.0276	14.2281	.6952	.1076
R x V----------	3	.0348	.1230	2.7000	.2144	.0147	2.1311	.0000	.0162
Cuts (C)---------	2	1.7536**	55.7547**	126.4516**	2.1175	1.5211**	45.3690*	.0000	4.8784**
Regression----	1	--------	50.3142+	252.8073- **	3.9205+	2.4894+	27.6105	.0000	9.1633-
Deviations----	1	--------	61.1952**	.0959	.3144	.5528*	63.1275**	.0000	.5934**
C x V----------	6	.0318	2.8532*	3.3451	.7011*	.0875	9.5389	.0000	.0273
Regression----	1	--------	2.5941-	1.8804+	.5674-	.1594- *	1.9310+	.0000	.0165+
Deviations----	5	--------	2.9050*	3.6380	.7279*	.0731	11.0605	.0000	.0294
Error b----------	8	.0351	.6308	5.7763	.1949	.0248	4.3997	.0000	.0151

1/ * Significant at the 5-percent level; ** significant at the 1-percent level; + or - following the regression mean squares indicates the sign of the regression coefficient.

TABLE 25.--Mean squares from the analysis of variance of characters analyzed and regressions of saponin ("x" variable) with the other characters, Ashland Farm, Manhattan, Kans. (irrigated) 1/

Source	d.f.	Saponin	Protein	Crude fiber	Ash	Fat	N-free extract	Defoliation	Hay yield
Total----------	39	0.1964	1.5234	17.5297	0.0283	0.1623	12.8532	3.0455	0.2583
Replications (R)-	1	.0112	.4100	.0086	.1513	.0102	.0276	.0250	.1729
Varieties (V)----	4	1.0846**	1.0364*	13.5208*	.5230**	.1289	8.9455	5.3500**	.0425
Regression----	1	--------	.4270+	49.0956- **	.5945+	.2019+	27.5845+*	11.7485-	.0512+
Deviations----	3	--------	1.2395*	1.3292	.4991**	.1046	2.7324	3.2172**	.0396
R x V----------	4	.0122	.1375	2.0837	.0059	.0684	2.6160	.0250	.0173
Cuts (C)---------	3	.5090*	14.8630**	138.1207**	1.7063**	1.1519**	92.9631**	30.7583**	2.9235**
Regression----	1	--------	1.7757+	365.0466- **	.0378+	1.5351+	267.4619+ **	.1600+	6.4064-
Deviations----	2	--------	21.4066**	24.6578	2.5405**	.9604*	5.7136	46.0575**	1.1820**
C x V----------	12	.1239**	.4629	8.7636	.1798	.1055	5.3650	.3833**	.0688**
Regression----	1	--------	.2380+	10.7008-	.0002+	.0431-	9.1366+	.1094+	.0136-
Deviations----	11	--------	.4833	8.5875	.1962	.1111	5.0221	.4082**	.0738**
Error b----------	15	.0165	.2777	6.8470	.0853	.0539	7.4488	.0250	.0044

1/ * Significant at the 5-percent level; ** significant at the 1-percent level; + or - following regression mean squares indicates the sign of the regression coefficient.

TABLE 26.--Mean squares from the analysis of variance of characters analyzed and regressions of saponin ("x" variable) with the other characters, Ithaca, N. Y. 1/

Source	d.f.	Saponin	Protein	Crude fiber	Ash	Fat	N-free extract	Defoliation 2/	Hay yield 2/
Total----------	23	0.2366	7.3641	13.7269	0.5472	0.1518	6.0802	------	------
Replications (R)-	1	.0033	.2016	2.5610	.0210	.0433	1.4751	------	------
Varieties (V)----	3	.1019*	1.0995	1.2369	.1425*	.1437	3.5667	------	------
Regression----	1	--------	.6042-	.7149+	.1648-	.3723+ *	.0737-	------	------
Deviations----	2	--------	1.3472	1.4978	.1314*	.0294	5.3132	------	------
R x V----------	3	.0056	.9269	3.9367	.0122	.0209	1.8079	------	------
Cuts (C)---------	2	2.3852**	78.8178**	126.3967**	5.2584**	1.1886**	33.0867**	------	------
Regression----	1	--------	11.4580+	103.7867-	.0209+	.0000	44.4073+	------	------
Deviations----	1	--------	146.1775**	149.0066**	10.4959**	2.3771**	21.7660*	------	------
C x V----------	6	.0347	.7810**	4.8319	.2121*	.0714*	6.4893	------	------
Regression----	1	--------	.2593+	6.0412-	.3064+	.0043+	1.7670+	------	------
Deviations----	5	--------	.8853**	4.5900	.1932*	.0848*	7.4337	------	------
Error b----------	8	.0172	.0965	1.9816	.0389	.0188	2.1420	------	------

1/ * Significant at the 5-percent level; ** significant at the 1-percent level; + or - following regression mean squares indicates the sign of the regression coefficient.

2/ No data available.

TABLE 27.--Mean squares from the analysis of variance of characters analyzed and regressions of saponin ("x" variable) with the other characters, Medford, Oreg. 1/

Source	d.f.	Saponin	Protein	Crude fiber	Ash	Fat	N-free extract	Defoliation	Hay yield 2/
Total----------	15	0.6217	1.9889	16.6616	0.3159	0.1360	8.1713	0.1167	-----
Replications (R)-	1	.0003	1.7490	3.4970	.2352	.0036	.9555	.0000	-----
Varieties (V)----	3	.2067*	.3547	6.7517	.1147	.0082	4.2194	.2500	-----
Regression----	1	--------	.4036+	17.2548-	.0182-	.0168+	12.4764+ **	.6760+	-----
Deviations----	2	--------	.3303	1.5001	.1629	.0040	.0909	.0370	-----
R x V----------	3	.0076	.2469	5.4905	.5150	.0049	5.5586	.0000	-----
Cuts (C)---------	1	8.4973**	24.0345**	162.3076**	.7056	1.6770**	54.5752*	.2500	-----
C x V----------	3	.0415	.5439	10.3347	.5473*	.0859	8.2408	.2500	-----
Regression----	1	--------	.2432+	20.1811+	.1449-	.1130+	16.3194+	.1868-	-----
Deviations----	2	--------	.6942	5.4114	.7484*	.0724	4.2015	.2816	-----
Error b----------	4	.0150	.1535	4.0973	.0668	.0156	3.2455	.0000	-----

1/ * Significant at the 5-percent level; ** significant at the 1-percent level; + or - following the regression mean squares indicates the sign of the regression coefficient.

2/ No data available.

TABLE 28.--Mean squares from the analysis of variance of characters analyzed and regressions of saponin ("x" variable) with the other characters, Davis, Calif. 1/

Source	d.f.	Saponin	Protein	Crude fiber	Ash	Fat	N-free extract	Defoliation 2/	Hay yield
Total----------	39	0.1679	5.3266	14.7064	0.3658	0.1161	7.8560	-----	0.0606
Replications (R)-	1	.3331	115.0566	318.1523	4.2250	.1782	17.8089	-----	.0255
Varieties (V)----	3	1.5599**	7.3867	9.7022	.4685	.2296	7.6506	-----	.0894
Regression----	1	--------	11.2374+	24.3533-	1.0232+	.0430-	.0217+	-----	.0739-
Deviations----	2	--------	5.4613	2.3767	.1911	.3230*	11.4651	-----	.0971
R x V----------	3	.0040	1.1378	2.9328	.4408	.0294	3.7671	-----	.0421
Cuts (C)---------	4	.0735	8.6897**	9.2653	.8718**	.0387	8.1462	-----	.3701**
Regression----	1	--------	3.2996+	11.9602+	.3344-	.1171-	19.9657-	-----	1.4062+ **
Deviations----	3	--------	10.4864**	8.3670	1.0509**	.0125	4.2064	-----	.0247
C x V----------	12	.0778**	1.3108	8.1487	.1523	.2396**	11.3187	-----	.0264*
Regression----	1	--------	.5492+	22.8129-	.2400-	.2148-	21.0810+	-----	.0015-
Deviations----	11	--------	1.3800	6.8156	.1444	.2419**	10.4312	-----	.0286*
Error b----------	16	.0185	1.0387	5.1654	.1250	.0339	5.3696	-----	.0093

1/ * Significant at the 5-percent level; ** significant at the 1-percent level; + or - following regression mean squares indicates the sign of the regression coefficient.

2/ No data available.

TABLE 29.--Mean squares from the analysis of variance of percentage of chemical constituents in alfalfa with the regressions of saponin on the other characters 1/

Source of variation	d.f.	Saponin	Protein	Crude fiber	Ash	Fat	N-free extract
Locations (L)----------	7	0.7255**	72.5332**	93.5924**	24.7923**	2.4156**	127.7384**
Regression---------	1		120.8237+	1.1058+	5.6838-	4.5785-	55.8721-
Deviations---------	6		63.2492**	109.0069**	27.9846**	2.0551**	139.7161**
Replications in locations (R in L)--	8	.0284	.6433	3.8256	.1390	.0252	5.0035
Varieties (V)---------	3	1.4297**	4.1195**	8.6759	.4473	.3601**	4.6009
Regression---------	1		5.4248+	23.1019-	.2153+	1.0561+ **	1.2245+
Deviations---------	2		3.4668*	1.4629	.5632	.0121	6.2980
V x L----------	21	.0770**	.7026	7.5568**	.1955	.0564	7.5955**
Regression---------	1		.5142+	5.1714-	.2682+	.1500+	.5450+
Deviations---------	20		.7121	7.6760**	.1919	.0517	7.9480**
V x R in L----------	24	.0151	.6392*	2.5284	.1734*	.0711	2.3094
Cuttings (C)----------	2	3.7909**	132.2908**	655.3796**	5.9549	1.6030	174.9494*
Regression---------	1		215.4930+ **	1310.4493- **	10.8498+	2.9906+	268.9600+ *
Deviations---------	1		49.0885	.3100	1.0600	.2153	80.9378
C x L----------	14	.5589**	17.2610**	35.3494**	2.7116**	1.1383**	31.8639**
Regression---------	1		.6605-	118.4236-	.2844+	.7850+	105.6788+
Deviations---------	13		18.5380**	28.9591**	2.8923**	1.1654**	26.1858**
C x V----------	6	.0654	1.8980*	12.7621*	.9223**	.1236	4.6346
Regression---------	1		8.9522+ **	21.2940-	3.5626+ **	.2656+	.6117-
Deviations---------	5		.4871	11.0557*	.3942	.0952	5.4392
C x V x L----------	42	.0541**	.7159**	4.0349	.1986**	.0714	4.2967
Regression---------	1		.2966+	.0390-	.0902-	.0028-	.00002
Deviations---------	41		.7262**	4.1323	.2013**	.0731	4.4015
Error----------	64	.0196	.3646	3.6076	.0990	.0487	3.6617

1/ * Significant at the 5-percent level; ** significant at the 1-percent level; + or - following the regression mean squares indicates the sign of the regression coefficient.

TABLE 30.--Mean squares from the analysis of saponin, defoliation, and yield with the regressions of saponin on yield and defoliation of alfalfa 1/

Source of variation	d.f.	Saponin	Defoliation	d.f.	Saponin	Hay yield
Locations (L)--------	5	0.8011**	20.8625**	6	0.8365**	2.7851*
Regression--------	1		25.1148+	1		5.5595-
Deviations--------	4		19.7994	5		2.2302*
Replications in locations (R in L)-	6	.0212	.0069	7	.0319	.3540**
Varieties (V)--------	3	1.0445**	3.9329**	3	1.3860**	.5177**
Regression--------	1		10.9603- **	1		1.3991+ **
Deviations--------	2		.4192	2		.0770
V x L----------	15	.0802**	.6773**	18	.0801	.0738**
Regression--------	1		.3166-	1		.5139- **
Deviations--------	14		.7031**	17		.0480
V x R in L----------	18	.0136	.0069	21	.0164	.0269
Cuttings (C)---------	2	3.2774**	13.1875	2	2.6889	17.2601**
Regression--------	1		2.8281-	1		34.3266- **
Deviations--------	1		23.5468	1		.1935
C x L----------	10	.4048**	9.0375	12	.4382	.7382**
Regression--------	1		3.9263-	1		.1909-
Deviations--------	9		9.6054**	11		.7880**
C x V----------	6	.0628	.3356	6	.0613	.1233**
Regression--------	1		.2047-	1		.3987- **
Deviations--------	5		.3618	5		.0683
C x V x L----------	30	.0679**	.1634*	36	.0580	.0286
Regression--------	1		.5068+	1		.0039-
Deviations--------	29		.1516*	35		.0293
Error----------	48	.0187	.0070	56	.0199	.0215

1/ * Significant at the 5-percent level; ** significant at the 1-percent level; + or - following the regression mean squares indicates the sign of the regression coefficient.